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The Effect of Rolled Oats on Cold Resistance in *Drosophila melanogaster*.

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Abstract

Changes in an organism's morphology, physiology, and behaviour can all affect its ability to withstand stress. Environmental stress can be influenced by diet and other external environmental factors. Dietary nutrition, both in terms of quality and quantity, affects resistance to stress. This study showed how rolled oats affects the cold tolerance of *Drosophila melanogaster*, which is cultivated on wheat cream agar media and mixed oats media. Result showed that, flies fed with oats were less resistant to cold than those fed wheat cream agar media suggesting that oats media does not provide any extra nourishment to tolerate cold than wheat cream agar media. In all diets, this study further reveals that female flies were more resistant to cold than male. Furthermore, compared to mated male, unmated male flies showed more cold resistance and mated female flies were more resistant to cold than unmated female flies. Thus, supplementing oats in culture media impact negatively by decreasing cold resistance in *D. melanogaster*.

Keywords: *Drosophila melanogaster*, oats media, diet, cold resistance, mated, unmated

Introduction

In insects, the nutritional content of their diet affects fitness-related traits as growth, immune response, stress tolerance, fertility, and longevity (Burger *et al*., 2007). Insect distribution and abundance can be impacted by frequent shifts in their surrounding environmental conditions. Changes in an organism's morphology, physiology, and behaviour can all affect its ability to withstand stress. The distribution and abundance of insects, particularly small ectotherms such as *Drosophila*, are significantly influenced by their ability to resist cold. Phenotypes related to organismal stress resistance, including thermal tolerance traits, are key determinants of ecological success and evolutionary potential in varying climates (Sinclair *et al*., 2003; Chown & Nicolson, 2004).These flies are significantly influenced by temperature acclimation. Due to the wide variation in resistance type and level within and between species (Stanley S.M, Parsons P.A, 1980; Kimura M.T, 1988). Reproduction and survival resources are systematically divided by life cycle approaches, and a variety of internal and external factors influence each's relative importance (Stearns S.C, 1992, 2000; Roff D.A, 2002). Because several of these factors—such as age, density, and reproductive status—change over the course of an organism's life, different allocations will be favoured via selection at different times or under different circumstances. As a result, organisms usually adapt by plastically altering the relative distribution of resources in response to environmental cues, especially when those changes are predictable (Scheiner S.M, 1993).

By altering energy metabolism, cryoprotectant synthesis, membrane composition, and oxidative stress management, diet and nutritional availability have a major impact on *Drosophila*'s ability to withstand cold. According to Colinet*et al*. (2013), trehalose sugar stabilizes proteins and membranes when exposed to cold, acting as a cryoprotectant. Another important factor is lipid composition, which is influenced by dietary fatty acids. According to Overgaard *et al*. (2005), flies that have adapted to cold temperatures exhibit higher percentages of unsaturated fatty acids in their membranes, which preserves membrane fluidity and functionality at low temperatures. Furthermore, by boosting metabolic efficiency and perhaps strengthening stress response pathways, moderate calorie restriction can increase overall stress resistance, including cold tolerance (Chippindale *et al*., 1996). Antioxidants and micronutrients are also important in addition to macronutrients. Dietary antioxidants like vitamins C and E can lessen the damage caused by cold exposure, which causes oxidative stress (Radyuk *et al.,* 2009). When taken as a whole, meal composition not only supports basic metabolism but also enhances *Drosophila*'s physiological resistance to thermal stress.

Due of variations in physiology, energy storage, and stress-response gene expression, male and female *Drosophila* react differently to cold environments. As indicated by quicker recovery times from chill coma and greater post-cold survival rates, females generally exhibit greater cold tolerance than males (Andersen *et al*., 2015). This distinction is partly explained by the fact that females have larger stores of lipids and glycogen, which are essential for preserving energy balance both during and after cold stress. These stores also help to produce cryoprotective substances like trehalose, which stabilize cellular structures in the face of extreme temperatures (Colinet*et al*., 2013).According to Zhang *et al*. (2015), females have a stronger cold stress response than males as they tend to upregulate genes linked to detoxification, immunological response, and metabolic balance more than males do. Furthermore, females may exhibit stronger or longer-lasting expression of heat shock proteins (Hsps), which aid in protein stabilization during temperature swings, which adds to their increased resilience (Sorensen *et al*., 2005). Environmental factors and population history, however, can affect the degree and direction of sex differences in cold tolerance. For instance, Rajpurohit and Schmidt (2016) found that males from particular populations showed similar or higher levels of cold resistance under acclimation regimes.Males may put greater effort into immediate mating activities, possibly at the expense of stress resistance, whilst females emphasize survival and future reproduction. These patterns may also represent sex-specific life-history trade-offs (Norry *et al*., 2008). Sexual dimorphism along with physiology impacted by varying thermal conditions has a key role in evolutionary and ecological implications specifically in a dynamic environment.

In *D. melanogaster*, mating status has a major impact on cold stress responses; mated and unmated flies exhibit different physiological responses to low temperatures (Rinehart *et al*., 2000; Overgaard *et al*., 2007). Interestingly, mated females typically have higher cold tolerance than unmated females, a trait associated with a number of interconnected physiological and biochemical variables (Rajpurohit & Schmidt, 2016). A series of alterations in female metabolism, hormone signaling, and gene expression are brought about by mating, and these changes collectively improve stress resilience (Wigby& Chapman, 2005; Harshman & Zera, 2007; Soller *et al*., 2001). In order to survive and recover from cold stress, mated females, for example, exhibit increased amounts of energy reserves, such as lipids and glycogen (Fricke *et al*., 2009). Seminal fluid proteins (SFPs) transported from men during mating induce the activation of genes involved in food storage and metabolism, contributing to these enhanced reserves (Gioti *et al*., 2012).Furthermore, the reproductive drive to support egg production causes mated females to frequently show enhanced food consumption, which raises nutritional intake and may result in a greater accumulation of cryoprotective chemicals such trehalose (Wong *et al*., 2009). The distinction between mated and unmated individuals is less noticeable but still exists in males. According to some research, mated males may have a lower tolerance to cold because of the energy trade-offs involved in copulation and wooing, which can drain energy stores (Pitnick*et al*., 1995). A sex-specific and resource-dependent response to reproductive activity and environmental stress is thus highlighted by the fact that, although mating increases cold tolerance in females through metabolic and molecular modifications, it may only marginally or barely affect males.

Oats are one of the ancient grains and food crops that are farmed and eaten all over the world. Among cereal crops, oats (*Avena sativa L.*) are unique since they include a range of nutrients like carbohydrates, dietary soluble fiber, balanced protein, lipids, different phenolic compounds, vitamins, and minerals (Joyce *et al*, 2019)that can be used for medicine, cosmetics, animal feed, and human consumption (Butt *et al*., 2008; Varma *et al*., 2016). As people become more conscious of the need of eating a healthy diet, businesses and scientists are becoming increasingly interested in oats. Beta-glucan, the primary active component of oats, has been demonstrated to have cholesterol-lowering and antidiabetic effects (Wood, 2007; Whitehead *et al*., 2014). Additionally, eating oats helps reduce atherosclerosis, dermatitis, and several types of cancer (Butt *et al*., 2008; Chu *et al*., 2013; Malkki, 2004).Oats are currently ingested by many fitness enthusiasts, especially athletes and obese people, due to their beneficial nutritional and physiological effects.

Since, there is no published data on how eating oats impacts an organism's ability to withstand colder temperature. Determining how oats impact *D. melanogaster*resistance to cold is the aim of this study.

Materials and Methods

Rolled Oats was purchased through Swiggy instamart app, Mysuru. This oat product was used to prepare the experiment media.

Establishment of Stock

D. melanogaster flies (O K Strain) were collected from the *Drosophila* stock centre, Dept. of studies in zoology, University of Mysore, Manasagangotri, Mysuru. The flies were cultured in glass bottles with Wheat cream agar media. Flies were maintained in laboratory conditions with 22±1°Ctemperature, 12:12 light and dark cycle and humidity level of approximately 70% RH.

Establishment of Experimental Stock

Flies obtained from the above-mentioned stocks were used to establish experimental cultures in various media.

Wheat Cream Agar Media (Control Media):

100g of jaggery, 100g of Rava powder (sooji)and 10g of agar in 1L of boiling distilled water and 7.5ml of propionic acid.

Mixed oats media:

25% Oats Media: 100g of jaggery, 75g of sooji, 25g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

50% Oats Media: 100g of jaggery, 50g of sooji, 50g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

75% Oats Media: 100g of jaggery, 25g of sooji, 75g of Rolled Oats powder and 10g of agar in 1000ml of boiling distilled water and 7.5ml of propionic acid.

These flies were maintained under the laboratory condition mentioned above and used to study cold resistance in *D. melanogaster.*

Experimental Procedure:

Both unmated and mated five-day-old flies were taken from the control and mixed oats media to investigate starvation resistance. Fifteen male and female flies from each of the control, 25%, 50%, and 75% mixed media were placed in empty vials, each containing five flies, and sealed with cotton. These vials were maintained in a laboratory setting, and each fly's resistance to cold was noted every hour until its death. The tests for mated and unmated flies were conducted separately.

Results

Fig. 1 shows the mean and standard error value of cold resistance in unmated male and female flies raised in 25%, 50%, and 75% oat media, as well as in wheat cream agar medium. Cold resistance was higher in wheat cream agar media, lower in 25% and 50% oat media, and least in 75% oat media, as per the findings. Additionally, these results demonstrated that in all given diets, unmated female flies were more resistant to cold than unmated male flies. The two-way ANOVA and Tukey's Post hoc test on the cold resistance data revealed a significant difference in the amount of time taken for unmated male and female flies raised on a mixedand control diet to survive in cold temperatures.Significant difference in cold resistance was observed between treatment. However, there was no significant difference in cold resistance between treatment and sex.

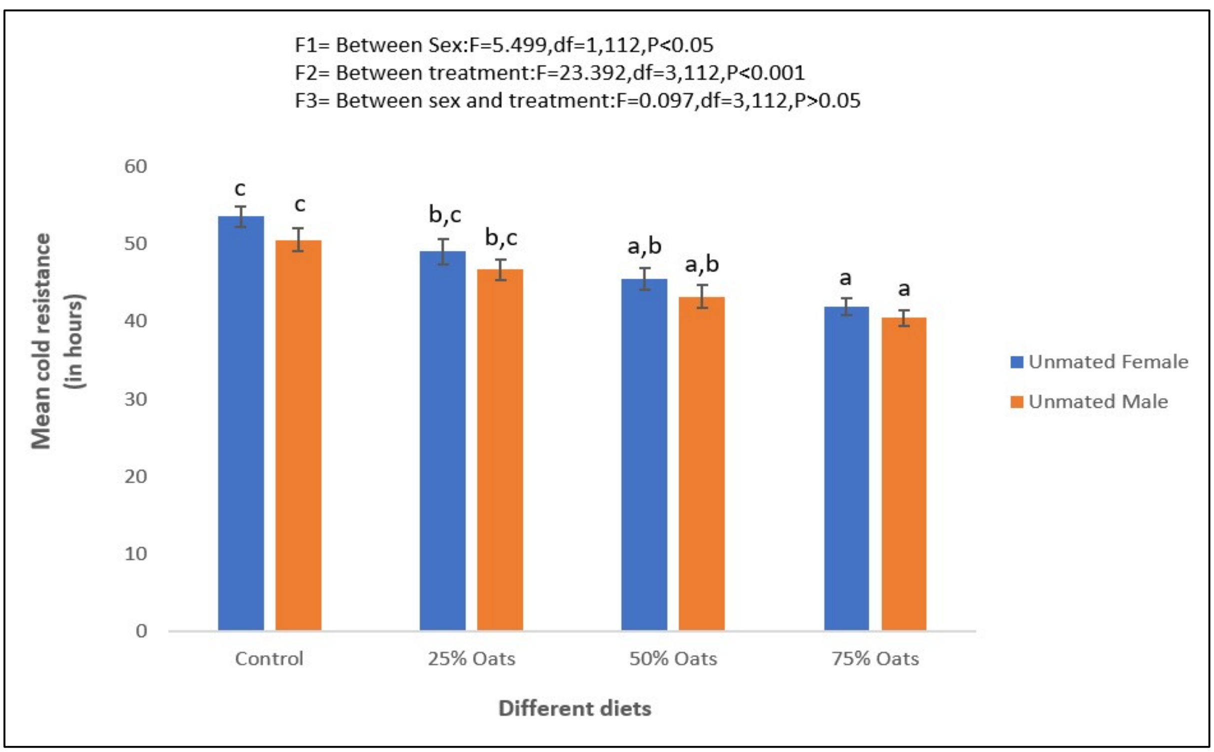

Fig 1: Effect of oats on cold resistance in unmated female and unmated female of *Drosophila melanogaster.*(The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Fig. 2 shows the mean and standard error value of cold resistance in mated male and mated female flies cultured in Wheat cream agar media and mixed oats media. The flies cultured in wheat cream agar media showed highest cold resistance when compared to25%, 50% and 75% oats media. Female flies exhibit higher cold resistance compared to male flies across all diets. The above cold resistance data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significant variation in cold resistance by mated male and female flies cultured in control and oats media of different concentrations. However, insignificant variation in cold resistance was noticed in interaction betweensex& treatment. Tukey's post hoc test showed significant variation in cold resistance in mated male and female flies of wheat cream agar and 50% oats media.

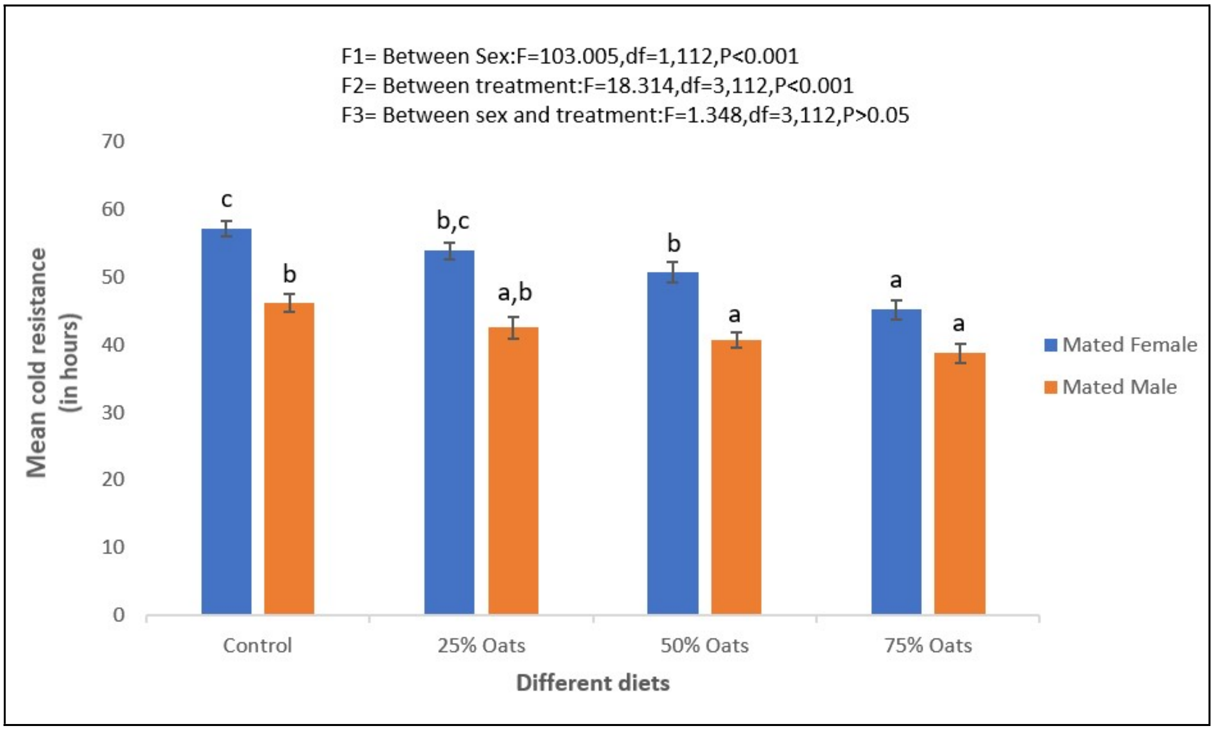

Fig 2: Effect of Oats on cold resistance in mated female and mated male of *Drosophila melanogaster*.(The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Fig. 3 shows the mean and standard error values of cold resistance in unmated and mated female flies grown in Wheat cream agar and mixed oats media. The results showed higher cold resistance in mated female fliesthan unmated females across all diets.Cold resistance in mixed oats media reduced significantly compared to control media. The above results were subjected to two-way ANOVA followed by Tukey's Post hoc test, which revealed significant variance in cold resistance across sexes and treatment. However, no significant change in cold resistance was observed across the sex & treatment.

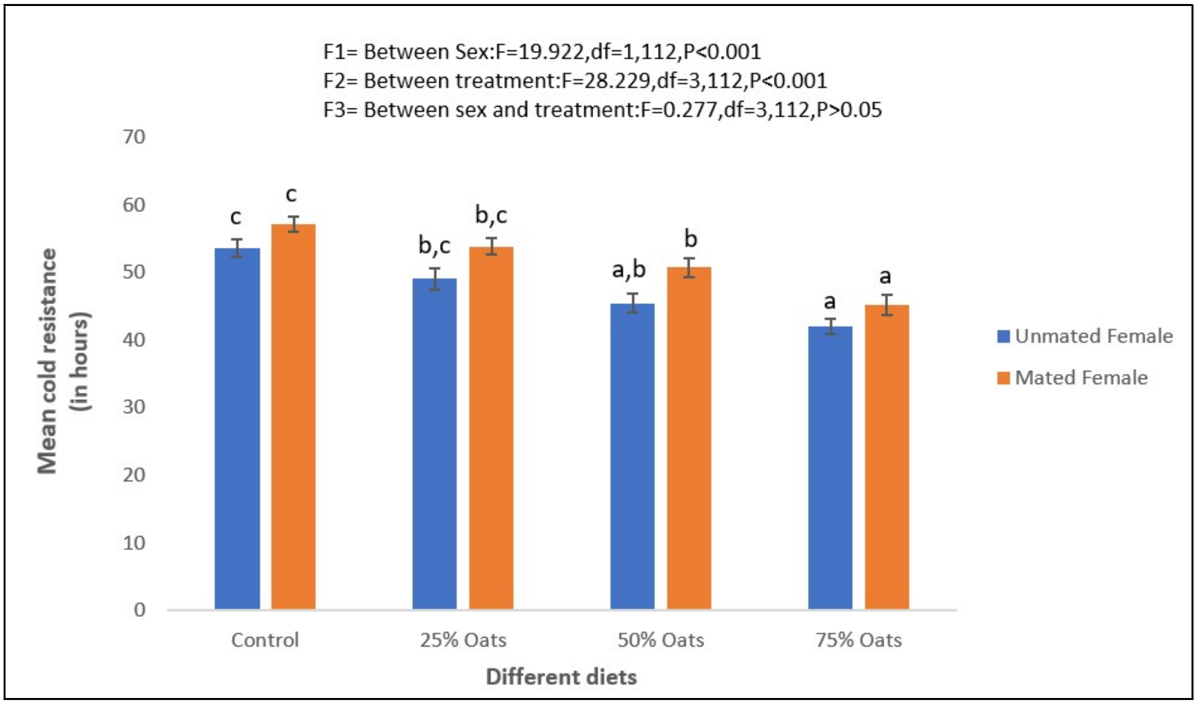

Fig 3: Effect of Oats on cold resistance in unmated female and mated female of *Drosophila melanogaster*.(The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Fig. 4 shows the mean and standard error of cold resistance in unmated and mated male flies cultured in Wheat cream agar and mixed oats media. When compared to other diets, flies fed on wheat cream agar media exhibited the highest cold resistance. In all the diets, the results indicated that unmated male flies were more resistant to cold than mated male. The above data was subjected to two-way ANOVA followed by Tukey's Post hoc test, which revealed significant variations in cold resistance between diets and between sexes. In addition, insignificant variation in cold resistance was observed in the interaction between sex and treatment. Tukey's post hoc test showed significant variation in cold resistance in unmated and mated males flies of wheat cream agar and mixed oats media.

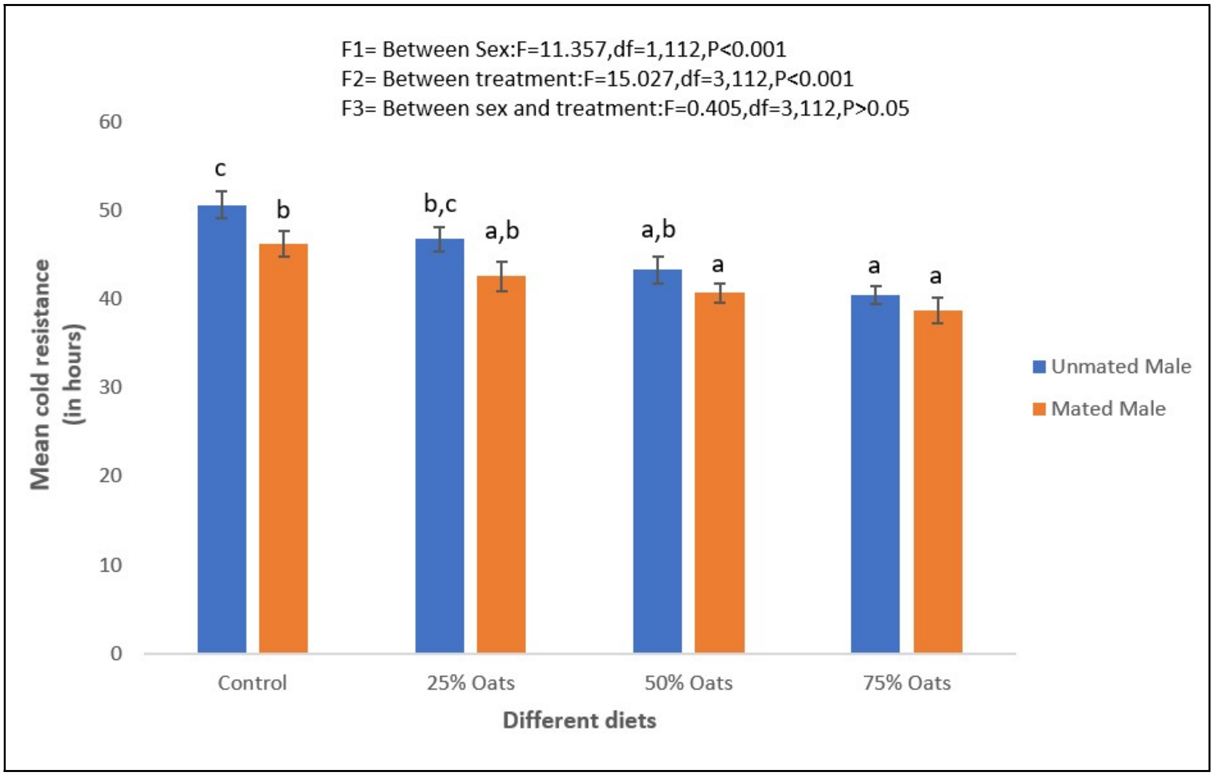

Fig 4: Effect of Oats on cold resistance in unmated male and mated male of *Drosophila melanogaster*.(The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test).

Discussion

The ability of insects to adapt to and cope with climate change has led to extensive research on stress-related traits in insects and other creatures. Variations in sex, diet, and genetic makeup can all affect an organism's ability to withstand thermal stress. Cold resistance in insects is influenced by food composition and availability. The results of this study (Fig. 1-4) show that flies fed with wheat cream agar media were more tolerant of cold, while flies fed with 25% and 50% mixed oats media exhibit significantly lesser levels of cold resistance and flies cultured in 75% oats media showed least levels of cold resistance. This implies that variations in cold resistance were influenced by the quantity and quality of nutrients that were available in the diet. Increased dietary fibre contents with less calorific value of oats does not provide sufficient energy*D. melanogaster* needs to tolerate cold. This implies that the flies' varying degrees of cold tolerance were influenced by the nutrient availability and quality of the diet. Selection for cold tolerance has been shown to increase lipid levels (Chen and Walker, 1994) whereas increased oat content decreases cholesterol levels in mammals (Anderson, *et al* 1990; Davidson, *et al*, 1991; Kirby, *et al*, 1981; Shinnick, *et al*, 1990; Guo, *et al*, 2014; Tong, *et al*, 2015; Braaten, *et al*, 1994; Hicks, *et al*, 1995) and lowered lipid levels in nematode model- *C. elegans* (Chenfei Gao, *et al*, 2015). Additionally, Kimura (1982) found that the buildup of haemolymph carbohydrates has been connected to *Drosophila's* development of cold tolerance. It was also discovered that a higher concentration of sugars in the flies' diet had a detrimental effect on their capacity to tolerate cold temperatures Colinet, et, al, (2013). Solubility of OBG, rather that amount is a driving component to decrease cholesterol (Beer *et al* 1995). Since the provided oat diet has low level of sugar and lipid constituents which resulted in lesser cold tolerance in the flies.

Sexual dimorphism in relation to cold resistance is likewise supported by our results. In most of the given diets, the results (Fig. 1 and 2) demonstrate that female flies were more resistant to cold than male flies. This implies that the ways in which males and females react to cold differ in terms of gene expression changes, indicating that they primarily employ the same processes to alter their physiology. Numerous studies have also indicated that both sexes' cold resistance is influenced by their dietary composition. Males consume body lipids as an energy source during starvation, but females use both glycogen and body lipids, which are typically stored in larger quantities, to meet energy demands under nutrient-deprived conditions (Zwaan *et al*., 1991; Hoffmann & Harshman, 1999). Increased energy storage or a slower rate of decomposition of these reserves are associated with improved resistance to starvation in females (Hoffmann and Harshman, 1999; Rion and Kawecki, 2007; Gibbs and Reynolds, 2012). According to this study, females were more tolerant to cold than male in all the diets which can be accounted to better utilisation of nutrition in females compared to males during stress. Due to proportional differences in the costs of life or reproduction or differences in the regulatory architecture, it may differ across the sexes (Grath and Parsch, 2016; Mank, 2017).

Furthermore, we found that the flies' ability to withstand cold varies with respect to mating, and that sexual differences also impact the flies' ability to withstand heat stress. Fig. 3 shows that the mated females were more resistant to cold than the unmated (virgins) females. Our study's results support those of Goenaga *et al*. (2012), who also showed that mated females exhibit higher stress tolerance than virgins in their research on *D. melanogaster*. Carvalho *et al*. (2006) and Ravi Ram and Wolfner (2007) have shown that female *Drosophila* increase their food intake after mating, which could offer a physiological explanation for the differences in stress resistance between mated and virgin females. Furthermore, the cause can potentially be related to the nuptial gifts that males give to females during mating. In particular, seminal gland proteins are transmitted to females during copulation, whereas accessory gland proteins are transferred by males during mating. Mated females may be more resistant to cold than virgins for this reason. Our results allow us to explain why the mating condition helps *D. melanogaster*'s physiological behavior, allowing both sexes to withstand and adapt to the cold. Fig. 4 shows highest cold resistance in virgin males compared to mated males. Unmated male *D. melanogaster* frequently has higher cold tolerance than mated ones because of variations in energy expenditure, physiological trade-offs, and stress responses related to mating behavior. In addition to copulation, mating involves persistent activities like courtship, mate guarding, and the generation of energetically costly ejaculate components such as seminal fluid proteins, which impose large energetic costs on males (Pitnick *et al*., 1995; Wigby *et al*., 2009). Critical energy reserves, such as lipids, glycogen, and carbohydrates—necessary for sustaining cellular function and withstanding cold exposure—may be depleted by these energetic demands.

Conclusion

Thus, we can infer from this study that rolled oats reduced cold tolerance in *D. melanogaster*. Additionally, in every diet examined, female flies exhibited noticeably higher levels of cold resistance than male flies. Also, compared to unmated female flies,mated females showed increased cold resistance; unmated male flies were more resistant to cold than mated male flies.

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